

Summary of Call with North Dakota DEQ and EPA regarding the state's fish and water sampling plan February 17, 2021

Attendees: NDDEQ: Aaron Larson, Joe Nett, Jim Quamstrom, Todd Ussatis, Pete Wax, Joshua Wert
EPA HQ: Joe Beaman, Erica Fleisig, Karen Kessler; EPA R8: Josh Baker, Andrew Todd, Holly Wirick

Pete opened the meeting explaining the principal reason for the call; essentially the state wants to make sure they collect data that's functional and use their time effectively in this process. He wants to make sure that when they put their QAPP together that the sampling protocol and the analytical procedures are appropriate. Our timing is going to be summer through late fall. Does that seem appropriate for the wadeable rivers and streams work?

Karen: it looks like you're planning to collect whole fish for these waters?

Pete: It's something we discussed earlier today. During the collection process we end up getting some larger animals when shocking. We haven't decided how we're going to deal with those yet but can talk about it now.

Karen: Let's back up a little bit and look at some of the things you guys listed in terms of things that you're collecting for water analysis. Is the main purpose of the sampling specifically to develop the selenium criterion or are you looking to accomplish multiple goals with the sampling?

Pete: multiple goals.

Karen: if you could let us know what all the different purposes are then maybe we can do our best to combine them all so we're hitting all the things that need to be done.

Pete: The fish would be collected in conjunction with our biological work that's being done on the streams. Joshua would be better at explaining all of the things that we collect, but we do ecoregion-wide across the state so I think we end up getting most of the state in 3 years – is that right Josh?

Josh: We are on a 5-year rotation –and that includes the national rivers and streams assessment so 2 years going to NRSA and then we go into Red River, James River, Souris River, and then out west. The sampling purpose of it is that we do 20 sites per year if we can get more in, we try to do more. With the smaller program that we have and all the other projects going on and then – the sites we're choosing for our IBI program are going to be the sites that we are going to use are the sites that we are going to use to collect the selenium data. Along with water chem, PHab, and a bunch of other stuff that we do.

Karen: So the goal of the selenium is to do a statewide value, correct Pete?

Pete: Right, but we start at Ecoregion 46 which represents – from the surface area - probably around one-third of the state.

Karen: So would the goal be to collect in all of the ecoregions over those 5 years and then look at proposing or picking up ecoregions as you have data for the different ecoregions?

Pete: I was hoping the results would drive that process. We could also do it by ecoregion if we get enough data where we could maybe write per ecoregion and it may vary per ecoregion as well so it might be an appropriate way of looking at the standard.

Karen: I just want to make sure you're not looking to apply the data from ecoregion 46 across the state without doing some sort of verification that that would be an appropriate thing to do.

Pete: Correct. I see we have mercury here; we're going to collect mercury, but that's not part of this discussion for today.

Karen: How are these sites selected for your IBI program? Is it a randomized selection or is it sites you go back to every year? How are they representative of the overall ecoregion 46?

Aaron: Originally these sites were targeted for reference and disturbed sites that we use for our biological indicators to refine fish and macroinvertebrate IBIs. This year Josh will be hitting 20 locations that we have visited before to try and refine those indices a bit further. As he visits those sites - we've been discussing this selenium topic for quite a while - and were hoping while he's out sampling for fish he could collect the fish tissue as well at those locations so we could get as much data as possible while our biological crew is out in the field.

Karen: Do you have particular areas where you know there are selenium issues? Would those be captured at these sites?

Aaron: Pete, do you know of any selenium hot spots?

Pete: This area probably does not have an issue with selenium. There are two areas within the state - they are in the northeastern corner of the state and the southwestern part of the state where you have a particular geological formation that's exposed. It's odd that they are on opposite ends of the state, but they are. They have naturally occurring clays that have selenium in them so when we have precipitation events, selenium does go up. Aaron should be able to pull that information from the 303(d) list because they do show up there. They're not anthropogenic sources, but they are sources that exist in the state. So there is a range of selenium - it's temporary. We don't really have a lot in our systems normally but during those precipitation events we do have elevated amounts of selenium.

Karen: As you move into other ecoregions, just make sure that you're getting a couple sites from those areas with the particular exposure, but it sounds like that's not an issue right now for this ecoregion. Bringing it back to whether it's the right timing, it depends on what type of tissue you are going to be collecting and if it's whole fish, then you just want to make sure that you're avoiding the spawning periods for the fish that you're collecting. So you'll want to either grab a month before or a month after they're done spawning because that can affect the selenium levels in the females. Alternatively, you can go for egg/ovary, but that becomes a lot more challenging logistically, and I think given that you're trying to hit multiple targets with this data - and Joe - feel free to speak if you disagree, but I would think doing whole fish probably would be a better option for you in order to have the data useful for multiple things.

Joe B: I concur. Just looking at your species list the cyprinids are indeterminate - they spawn a bit more broadly across a range of time vs. black bullhead - they're a fairly early spawner. Common carp is a batch spawner, I think white suckers are also a batch spawner but the other fish are a little bit indeterminate and they'll spawn more broadly across time once they come into reproductive maturity. One other question I have with regard to the stream sampling. Do you plan on collecting water concurrently with the tissue? It's a little bit more important to because the selenium that might be coming into the water body is probably a little bit more variable in lotic habitats, so it's probably more

important to capture the water samples as closely as possible to the fish tissue vs. a lake where you have more stable concentrations over time.

Aaron: Yes, there will be water quality samples collected in conjunction with the fish sampling and the tissue collection.

Karen: I think to help frame how to approach time of the year, it might be good to think about what fish species you want to actually target for this analysis. There are two elements of the fish that we think about when looking at what fish would be appropriate to sample for selenium; that's their sensitivity and their bioaccumulation potential which is going to be predominantly based on what they eat. The species that are more benthic-feeding and invertebrate-feeding are going to tend to have higher exposure to selenium, particularly if they're eating mollusks vs. algae-feeding species. You can go either more conservative and try and focus on your more bioaccumulating species, and that gives you pretty good coverage for all of your species, or you can try and balance the two and look at one set that are a little bit more sensitive but also still have a fairly high bioaccumulation potential. You can narrow down your list that way. Then you can look more specifically at what kind of spawners they are and that can help frame the appropriate time of year to go out and collect.

Pete: When talking with Josh and the sampling team, we were pretty much going to go out and grab everything we caught. So if we could get a large enough sample for them such as three species or three individuals plus and large enough weight, we could probably sort that kind of information out afterwards. We could tease those individual creatures out. Mother nature has to provide what we can get and I know that Josh and Aaron can speak more knowledgeably on what kind of animals we're going to get at the individual sites and how many species and types than I would.

Karen: That is one approach. You could capture everything that you have and look at the range of selenium that you're seeing and [pick out ones with greatest selenium concentration]. Knowing that you have limited resources, another approach could be to use a more targeted list and collect more samples of those targeted species which would give you better estimate of your variability at each site and give you a better estimate of what those concentrations are in the population. There are two different approaches... we're coming up on field season. My brain says, "do a pilot study," but nobody wants to do that.

Pete: to follow down that path I think we could do sort of a modification of that. If we would know which of those species are most likely to be bioaccumulators, would it be appropriate then to take multiple samples –because our time is so limited so if we do pick up a site and we end up with 50, 60 or 70 of a species that's like that, would it be advisable then – or do you think it would be useful to make multiple collections of those – maybe sort them out by size so we'd have more samples of that particular species?

Karen: The more samples you have, the better estimate you get of what that true concentration is at each site in the population. You're going to get a better estimate than if you only collect one or two of those fish. So if you're collecting 3-5 composites of those particular species, I think that gives you better data overall for your development of this criteria. Joe feel free to add on if you have thoughts on any of this.

Joe B: Just to add, depending on the species available at each site, if you're able to capture a large number of fish obviously you have a more robust estimate with a larger composite. If you don't capture

a lot of fish obviously smaller composites, and capturing a couple of species especially if you know their bioaccumulation potential. We're talking generally about the species you have – looking at insectivores like darters and stickleback – or omnivores that have more macroinvertebrates in their diet than omnivores that are more [algae-consumers] like carp and suckers so I think it depends. Like you said, you're bound by what mother nature provides you, but knowing ahead of time what species you're likely to get and then target them will enable you to focus your sample collection for selenium analysis based on what you have and ideally having enough samples of one species so that you have a robust estimate not only of the average concentration but hopefully, a little of the variabilities. If you have very few fish, obviously, several small composites. If you have a lot of fish, several larger composites. It depends on logistics, time available to you as well as the amount of money you have to spend on samples.

Pete: We'll have to talk with Aaron and Joe, and maybe when you guys have that list of fish that we collected out of Ecoregion 46 in the previous investigations. Maybe out of that list you could mark the ideal fish for that type of work so that Josh can have that in-hand when he's in the field.

Joe B: I'd be happy to go through the list and look at their diets and help prioritize for analysis.

Josh: for some of the fish that we might potentially be targeting I'm just looking at some of the data that we collected previously in Ecoregion 46 and some of the species, and we might not have high enough densities when we're going out to sample. We might only catch 2 or 4. If we don't meet that 50 – 100 or 20 grams for our sample, is it something that if we want to be opportunistic when we're out sampling, can we pull another species that we're going to reach those goals to be able to sample, or are we just not going to bother?

Joe B: If you only had 2-4 fish individuals from a species and particularly if it was one of the highly desirable species, I would recommend if you could only get 50-100 grams and it's one composite, I would definitely take those fish and create a sample with them. Obviously, if you don't have enough weight to do a sample, it doesn't really matter what species you have. You're bound by your tissue weight limit to start with. It would be ideal to have multiple composites per species, but if you could only do one then do one. And then do other species like you recommended.

Pete: That's a good lead into the next 3 questions. I know that not getting the right method and the right collections have been things I've seen other states stumble over. We want to make sure we get those correct. Jim and Todd are on the phone making sure we get the preferred or the proper analytical methods for all the fish and for the water and making sure the fish are prepared correctly in the field. Do they need to be homogenized in the field? Do they need to be frozen right away? Can they come back on just ice and then prepared and frozen, and is there a time frame for that? Those sorts of questions.

Karen: I can give you my best answers that I know of for this, but I think talking specifically with labs is probably the best way to get answers about this. EPA Region 10 just ran a bunch of tissue samples for some folks in Montana and the way that they prepped their samples – and I can send this information in an email – was 3052 which was a Microwave-Assisted Acid Digestion and then they used 6020B which is Inductively Coupled Plasma Mass Spectrometry to do the selenium analysis in the tissue. The 6020B method is the one I see most people using. The other thing that I've heard from a lot of people is that when you're doing your dry weight analysis, that if you have the capability of doing freeze drying vs. oven drying, then to go with the freeze drying method. It might be a more useful to get lab people to talk with the lab people. I can reach out to some of my counterparts and see if they can put you in touch

with Region 10 lab folks. They could potentially answer those questions better. In terms of water, I am not sure what is the preferred methodology. I know there are two EPA methods: 200.8 and 200.9; 200.8 is based on the information from the 1994 methods. It has a really high Method Detection Limit (MDL) so it doesn't seem ideal to use. 200.9 has a much lower MDL but it might be more useful to talk to a lab and see what methods they find are the best for it.

Joe B: I think that would be my recommendation. I would definitely check-in with labs to get their input. You want to make sure you have a low enough MDL to make sure you're not getting a lot of non-detects in your samples so you can calculate robust Bioaccumulation Factors (BAF).

Todd: I'm a chemist at the lab and historically we have used method 3050B which is actually soil digestion. My worry has always been no matter what you do when you handle a fish sample is you're going to end up with oils. Oils tend to coat the cones of the instrument and also the detector if it's not done right. But I like 3050B just because at the end you use hydrogen peroxide which is a great oxidizer which really tears things apart. It helps things get into solution and then after that I do use method 200.8 for my selenium but I use C-spring quartz nebulizers along with cyclonic chambers which a lot of people don't use – they like to use the scotts chambers which does not give you the detection level low enough to see what you need to see. Historically, I've done for the last 15-20 years of doing these types of samples and that's what I follow for doing selenium or actually any kind of heavy metals in the fish. Just a heads-up that's how I handle it.

Karen: I trust you. Probably talking to other lab people is probably the best way to go with this.

Pete: Maybe not looking at it necessarily from the method perspective, you can do selenium as a total recoverable or you can do it as a dissolved. Is there an advantage from one of dissolved over total? And what would it be? Our fish data has always had a low enough detection. Todd has done a wonderful job with the fish data in the past and we don't end up with non-detects in fish; but we do in water, so maybe we should concentrate more on the water issue. What would be better selenium analysis for the water, dissolved or total, and what would be a minimum or a maximum for a detection level in order to be able to draw the kind of relationships that we need?

Karen: I would probably say go dissolved. The criterion as written right now is a dissolved value for the water so I'd say go dissolved and then you can do the BAF to get a dissolved value for the state. In terms of detection limit, I think as low as is feasible is the best way to go. You want to make sure you're capturing actual values even for those sites that have really low concentrations. I'm not sure if you guys use an RL or PQL or anything like that but trying to account for that in terms of if you do some sort of adjustment to the MDL in terms of what you use for making decisions within the state. I think everyone has their own way of approaching that so I don't know if there's really a maximum detection limit, I think just as low as you can get is probably the best way to approach it – and reasonable – with resources.

Pete: As far as preparation goes, if we electroshock them in the field, they're generally alive. Do they need to be weighed, homogenized and put on dry ice in the field? Or can they be iced and 48 hours or 78 hours later, prepared and dry-iced? That's another question.

Karen: I believe they can be iced in the field and brought in. We have other people in our office who do a lot of sampling so I will ask them if they can send me what their field methods are but I do believe you're o.k. icing them in the field and then bringing them in. I think they can be frozen for up to six months. I

think you essentially want to make sure that you get your analysis done within six months of collection but I don't think there's any need to homogenize in the field and it would be a little bit cleaner and easier in the lab.

Pete: Then when you send that information we'd have an idea of the time frame because we do generally have dry ice in the field. They could be frozen in the field but then they would have to be frozen, then thawed, then homogenized, then frozen again and I'd think we'd want to try to avoid that if possible but there could be a fairly large time lag, too, between if we just iced them. Joshua, I'm not sure how long you stay in the field usually.

Josh: It just depends on how things line up. It could be two days – usually it's no more than three days. Or it could be day trips which wouldn't be an issue, but fish on ice overnight and not being frozen if that's o.k. we could do that. Or I could pick up dry ice, too, and bring that in the vehicle and that'll be fine, too, depending on the weather, for the three days.

Karen: I'll ask them about that. I'm not sure if they ever do any sort of collection when they're out more than one day at a time, but I'll see what they say.

Joe B: One of the biggest concerns about prolonged periods prior to processing is moisture loss because you have to calculate a dry weight. You want to make sure you get as accurate a percent moisture as possible from the fish. You can use surrogate species, but it's not advisable, and since you're going to be out there anyway, being able to capture that part of measurement accurately is important.

Pete: How would you prefer them, on dry ice, I'm assuming, once we've homogenized them?

Todd: Sure that would be fine because I'll put them in the freezer when I get them. In the past we'd actually try to do the freeze dry but I don't think we have that ability right now anymore.

Pete: On the water column samples that we'd be taking, we usually do both dissolved and total. Are there other elements or nutrients or those kinds of things you think would be useful to go along with that?

Karen: Not necessarily from a selenium perspective. It's more about what's useful to you guys in terms of doing your normal sampling. I think as good practice, I'd always grab temperature, conductivity, DO, etc. just so you have a sense of what's going on in the water; things you could get pretty easily with the YSI meter, but beyond that it's more what's important to you guys for your program.

Pete: The number of sites that'll be visited is 20 and then there'll be a certain number of repeats, I believe two, and if you add all that up and assume 10% or 12% QA/QC samples, you'd round up to 127. Does that look robust enough to do something with?

Karen: So it's 20 sites and the 100 is collecting 5 composites at each site?

Pete: Correct. And then they'd repeat two of those sites hopefully to get five more from each of those sites and then I just assumed that we would probably duplicate 10% of - every 10th sample of fish, and take another sample of composite for QA/QC. I questioned whether 10% is appropriate or do you do QA/QC for each site?

Karen: Generally, when we take samples, I'd have people doing sorting, 10% QA/QC is the line we'd use on stuff like that but I don't have as much experience with fish tissue sampling. I think starting off with the five composites is a good place to start for the site if you guys have any old data that can give us kind of a sense of if there's high variability at those sites or not. If they're not particularly variable then we'll probably be fine. It's always that question of if something weird is going on at the site and samples are all over the place, you might need more but if that's not going on then it sounds like you don't have any particular sources that might be causing big ranges in the selenium, then it's likely o.k. It's just hard to say without data.

Pete: I think we'll stick with the 10% unless somebody thinks of a reason not to. That's a pretty standard deal for us so I think it'll be easy for us to remember. So to roll back and look at what we've gone through here – 20 samples at each site. We can do multiple ones of individual species – we weren't sure if that was appropriate or not and it seems like it is and if we target ones that are most likely to give us good selenium information that would be better yet. So that takes care of the stream stuff. We're hoping to have a shocking boat delivered sometime this summer and we'd be able to then collect fish from lakes. And we would look to be doing lakes to complement the stream work. We'd be looking at lakes in Ecoregion 46 as well. So those would be larger animals in general because our lakes are fairly brutal and are generally stocked so I'm assuming we would probably catch a preponderance of game fish – northern pike, walleye, suckers, carp – a lot easier to take a plug or fillet than a whole fish on those kinds of creatures but I'm assuming that that would not be directly comparable. And ideally, is a whole fish a better sample for us than a fillet?

Joe B: Yes I think we've seen a whole fish provide a more accurate representation of selenium bioaccumulation in fish. One thing when we say whole fish you're looking at a 3 or 4 lb walleye – you're probably not talking about sticking a whole fish in the bass-o-matic. You're going to take representative subsamples of different tissues and weigh them and then weigh them against the whole body weight and then do some math to figure out concentrations. When you do take the whole fish it's definitely important to take the liver because the liver is the site of selenium detoxification as well as selenium metabolism in the fish so that's an important aspect of taking the whole fish. I think we can get back to you - there are protocols - we typically don't get into that part of it. I know FWS does whole fish for ecorisk purposes and they probably have a pretty standardized protocol for taking larger fish and sampling different aspects of the fish to get a whole fish sample so we could talk with some of our counterparts there and get back to you. I guess the second best option would be taking muscle. One question I do have on the species and the fish – when you're thinking about selenium – it most often manifests itself as a reproductive toxicant. It may also inhibit growth in fairly small young-of-the-year fish if there's a lot of selenium there. My question with regard to stocking vs. natural reproduction in these lakes where you do have some of these bigger game species. Is that mostly stocking or do you have natural reproduction of these species, because you'd want to target species that have natural reproduction on in the lake particularly, versus a fish that's being stocked and you don't have natural reproduction.

Josh: Depending on the body of water, we have a heavy propagation program - there's a lot of stocking. If we're trying to use this to meet multiple objectives, especially mercury, we're going to be looking at walleye but 90+% of these bodies of water are going to be stocked. Unless we look at certain data from Game & Fish when they do fall repro netting to try to see if there are some lakes we can target to see which ones are having some natural recruitment but most of them – besides our biggest bodies of water – Lake Sakakawea, Devils Lake, etc. I can't be 100% sure, but there's little natural recruitment.

Karen: If you can get that reproduction data that would probably be helpful to you. Would you guys have a sense, based on the length of the fish, of knowing how long those fish have been in the lake? Are they getting stocked as juveniles and then you can tell which ones have been there for several years? Another thing is you don't want to take a fish that was just put into that lake because it's not going to give you a good representation of how selenium has been incorporated into that fish if it's only been there for a short period of time.

Josh: We can probably look at that data from Game and Fish. I know they've done some studies from stocking in some of these district lakes for what those producers are, what those fish are consuming – some of those fish are growing very fast; three to five years. People are catching some decent-sized fish, so depending on where they are stocking fish – if it's a new lake, if it's an existing lake – I'm assuming depending on the lakes that Joe has chosen, they're going to be existing, established lakes most of the time. So we should be able to go along with some lakes maybe that they've pulled some older listing data to give us some age and size ranges to give us an idea of maybe some sizes to target.

Karen: Yes, that would be a good idea because you want to make sure that you're capturing that exposure.

Josh: Is there a certain time frame? If there is a lake where we know there is some natural recruitment, but of course we might be sampling some fish that might have been stocked. Should there be a certain age or class that we should be focusing on? Or as we're sampling, if we know an 8" walleye or bigger is going to be perfect, we'll only net those fish and bring them in the boat?

Karen: Unfortunately, no there's not a magic number; they're site-specific; my best guess is at least 1-3 years you'd want, particularly in lakes we see a lot slower accumulation and they can stay in the lakes longer, but that's just a very rough guess, unfortunately. I'd say 2 years + that they've been in the lake.

Joe B: You definitely want to get the larger fish that have been in the lake for a little bit longer. One question I do have regarding the food webs - and maybe Fish and Game have a better idea - I was thinking about whether they stock the forage fish. If we're getting really good growth rates, are you stocking forage fish also, or is it the natural reproduction from the forage fish that are naturally occurring there?

Josh: No – most of the time it's the natural reproduction – and that's just the district lakes that I'm talking about. They've been creating these fisheries all over the state. They go in and set some nets and see there's good forage base here. They throw in a certain species and then a couple years later they'll throw in another species and then they've got a great fishery that gets used locally. It gets hit hard.

Joe B: I was trying to think about selenium in terms of its toxicity. If you continually stock the water body and it's getting predation pressure, but you have good forage base, and it's naturally reproducing – that's great. If you do have a selenium issue, it may manifest itself first in the prey base rather than the predators that are being stocked. But it sounds like, at least anecdotally from I'm hearing, you're getting pretty good growth over 3-5 years in those fish. It might be good to just think about in a couple of water bodies, particularly if there are lakes that if you have potential for some selenium discharge, whether it's anthropogenic or natural, to get an idea of what the selenium is in the food web lower than the target species that you might collect for multiple purposes – like selenium and mercury.

Josh: For those larger species for our target per composite, would you guys still recommend around that 5, even though we will be way over the weight – most likely?

Joe B: I would say smaller composites and if you're focusing on one species and you have a lot of species, you'll get a better idea of the variability of the selenium if you take multiple small composites versus a couple larger composites that may dilute out the variability within a composite.

Josh: So it's multiple replicate composites, you're saying?

Joe B: Yes if you get a lot of fish maybe three per composite versus five per composite.

Pete thanked everyone for attending the call and asked folks to please stay in contact. The last thing on our list is a note that there might be some opportunity for some opportunistic sampling – both USFWS and the NDG&F do some population surveys and they do some contaminant work of their own where we might be able to get some ovum because they'd know exactly what they're pulling out of there.

Karen: As you guys start writing things up like QAPPs, we're happy to take a look at those and give you as much feedback as we can.

Pete: We have a very small lab and they're fantastic and I want to give a shout out to Jim and Todd for helping and showing up today and look forward to communicating with you guys again. Karen and Joe, we will be using you guys as a sounding board as we put together the QAPP for this. Appreciate your efforts today.

ND WQS Call Summary 2172021 re Selenium Sampling in 2021